Four bacterial soil populations characterized and compared by a factor analytical method

VERONICA SUNDMAN
Department of Microbiology, University of Helsinki, Helsinki, Finland
Received November 5, 1969

SUNDMAN, V. 1970. Four bacterial soil populations characterized and compared by a factor analytical method. Can. J. Microbiol. **16**: 455–464.

Bacterial populations from four adjacent sites with different soils were compared. The sites sampled were acid forest humus, grassland soil, and adjacent duplicates of open field soil. About a hundred random isolates from each of the four soil samples, 467 in all, were submitted to 44 two-state tests. The correlations between the tests were computed in terms of the test results obtained with all the isolates. Forty-two of the tests were accepted for further consideration. Their correlation matrix was factorized, and the first nine principal factors, covering 99% of the total estimated communality, were taken into account after varimax rotation.

With the aid of within/between population variance, the ecologically discriminating efficiency of the factors was determined. For qualitative description of the populations six significantly discriminating factors ($P < 0.1\%$) were used. These factors covered 56% of the total factor variance considered. The populations were characterized by the position in the 6-factor space of the point centroids (population means or "centers of gravity") of all the item points making up a population.

The six factors of ecologically discriminating efficiency were dominated by attributes on (1) oxidase, and nitrate reductase, (2) *Bacillus* characteristics, (3) protocatechuic acid utilization, (4) pH tolerance, (5) nutritional demands, and (6) pigment-correlated hydrolytic abilities.

The similarity or difference between the populations was expressed as the squared distance (d^2) between the population means in the selected discriminating 6-factor space. This parameter varied from 10 for the pair of duplicate field soil populations, to 172 between one of the field soil populations and the forest humus population.

Introduction

Bacterial soil populations have thus far mostly been described in terms of numbers and kinds of bacteria present. For such descriptions, sets of random isolates were characterized by a number of attributes, chosen to permit the classification of the isolates.

Such work is burdened by limitations of two kinds. *Firstly*: from the ecological point of view it might be more informative to know about the biochemical and physiological potentialities of the total population than to be able to denominate the individual component isolates. Therefore a descriptive numerical approach, which, making use of *all* the information gathered by the test battery applied, aims at characterizing the population as an entity, appears likely to be more profitable than the classical systematic approach, the goal of which is to classify the individual isolates. *Secondly*: hitherto only a small fraction of the total bacterial soil population is within the reach of the isolation methods, maximally about 1% of the living bacteria present in the soil (Prasad 1968). This makes it questionable whether the results of population description are reliable.

The numerical techniques applied in microbiology in recent years have contributed to the description of soil populations. Gyllenberg (1964), applying the method introduced by Hill *et al.* (1965), considered soil isolates as points in a three-dimensional Euclidian factor space. Sundman (1968*b*) developed the method further to comprise an arbitrary number of factors according to the amount of test variance to be covered, and showed with the aid of analysis of variance that some factor estimates were constant features of the bacterial soil populations, recognizable even after a soil inoculum had passed several monthly growth passages in moist sand under controlled laboratory conditions. Quadling (1967) introduced a two-stage principal component method for stepwise condensation of the information obtained with large sets of dichotomous tests, and Skyring and Quadling (1969) used this method for comparison of populations of soil bacteria derived from rhizosphere and nonrhizosphere soils in terms of clusters observed in the various populations.

The purpose of the numerical description of bacterial soil populations reported in this paper was threefold. Firstly, it was desired to rank the two-state tests used according to their efficiency in discriminating between bacterial populations derived from different soils, and thus to discover

the ecologically rather than taxonomically important tests. The second purpose was to describe the whole populations in terms of the tests applied, without any effort to define individual isolates. The third purpose was to obtain a simple numerical expression for the difference between populations, which would permit evaluation of the reproducibility of population description work, and with the help of which the ecological dynamics of bacterial soil populations could be studied.

Materials and Methods

Soil Samples

The investigation was confined to bacteria of four soil samples from neighboring sites, 5 to 50 m apart. The sites chosen were as identical as possible in respect of the underlying mineral soil, but different according to the plant cover, and were situated on a clay loam area where forest, grassland, and arable land meet. The samples were taken in May 1966. For details see Sundman (1969). Samples of about 10 kg soil were collected into sterilized plastic vessels from (1) acid forest humus from 0 to 10 cm under the litter cover of a mixed needle/broad leaf forest consisting mainly of Norwegian spruce; (2) grassland soil on an area used as pasture, from just beneath the coherent root layer, including the soil which was loosened when the turf was dashed against the sampling spade; (3) surface layer, 0–15 cm of open plowed field, where barley had been grown during the previous vegetation period; (4) a duplicate of the foregoing, taken 5 m from it. The pH values of the soil samples, as measured with a glass electrode in soil/water ($\frac{1}{3}$), and in 0.1% KCl added to the sticky point, respectively, are shown in Table I.

Isolation and Maintenance of the Bacteria

Immediately after the sampling, the main samples of about 10 kg were mixed aseptically by hand, and coarse particles were removed. Five 1-g subsamples from various parts of a main sample were pooled and added to 500 ml of sterile distilled water. This was done in quadruplicate. After homogenization with an Ultra-Turrax disintegrator, applied for 1 min, two successive hundredfold dilutions of each of the four suspensions were prepared with distilled water. These were thoroughly shaken by hand, and triplicates of 1 ml and 0.1 ml of the dilutions were immediately plated in soil extract agar termed *IM agar* (isolation medium of Taylor 1951), the soil extract of which originated from the same soil as the dilution to be plated. The plates were incubated at 21° for 14 days. Ten to 12 parallel plates, with 20 colonies or less in each, were inspected for each soil sample. The plate counts are shown in Table I. All colonies from the plates inspected were transferred as stab cultures to semisolid (0.3% agar) IM agar, the soil extract of which was prepared from equal parts of field soil and grassland soil. This was the universal base medium of most of the tests described below. A small number of the isolates from the forest soil required forest soil extract for growth. Those bacterial isolates which, in the routine examination for microscopic characters, were considered non-axenic were removed, as were isolates of streptomycetes. A total of 467 random bacterial isolates were included in the investigation. The distribution of these among the four soil samples is shown in Table I.

For maintenance, the isolates were transferred twice yearly in semisolid IM agar, and stored at 3° between transfers. The cultures were transferred maximally four times after isolation before they were submitted to any of the tests described below.

The Tests

For each isolate 44 attributes were recorded from a corresponding battery of tests, listed in Table II. Inocula for the tests were taken from young stab cultures (2 to 7 days old) in semisolid IM agar.

Microscopic characters, including Gram staining, presence of endospore, and permanently prevailing rod form were determined from IM agar slope cultures incubated at room temperature (23–26°) and examined, when visible growth appeared and 14 days later, as smears stained with Hucker's modification of Gram's method, and as water inclusion preparations with positive phase contrast equipment.

The presence of non-diffusible pigment and of water-soluble pigment was recorded whenever noted on any of the test media used.

All the following tests on enzymatic activity and growth requirements were performed at 28°. Acid production from carbohydrates after 3, 7, or 14 days, and growth under vaspar seal (Hugh and Leifson 1953) using semisolid IM agar as base medium and carbohydrate solutions sterilized by filtration. Dissolution of calcium phosphate

TABLE I

Number of isolates investigated, plate counts, and pH of soil samples

Sample	No. isolates	Plate count, millions per gram of fresh soil	pH (glass electrode)	
			Soil:water 1:3	Soil paste in 0.1% KCl
Acid forest humus	93	6.2	4.4	3.4
Grassland soil	148	16	6.2	5.5
Field soil, site 1	121	140	6.2	5.5
Field soil, site 2	105	170	6.3	5.9
Total	467			

TABLE II

Percentages of positive test results, and discriminative efficiency of tests, given as *chi-square* of the distribution, with uniform distribution in the four populations adopted as null hypothesis. In those cases where the distribution was too skew to allow a safe application of the χ^2 test (Moroney 1962) the data were pooled to form three or two populations, as indicated by the reduced number of degrees of freedom (d.f.)

		Populations					
				Field			
	Test	Forest humus $N=93$	Grass-land $N=148$	Site 1, $N=121$	Site 2, $N=105$	Total, $N=467$	χ^2 (d.f.=3)
1	Gram staining	59	62	69	59	62	3.7
2	Presence of endospore	10	2	2	1	4	13.6*** (d.f.=1)
2[a]	Permanently prevailing rod form	53	72	55	56	60	13.6**
3	Non-diffusible pigment	15	26	44	48	33	35.3***
4	Diffusible pigment	0	5	7	2	4	0.4 (d.f.=1)
5	Acid from glucose aerobically	69	60	64	64	63	2.1
6	" " mannose "	60	48	60	53	55	5.3
7	" " rhamnose "	41	42	48	43	44	1.5
8	" " xylose "	56	50	46	44	49	3.6
9	" " saccharose "	36	35	32	49	37	8.1*
10	" " maltose "	73	37	48	36	47	36.7***
11	" " cellobiose "	63	40	42	43	46	14.9**
12	" " glucose under vaspar seal	40	27	15	17	24	21.8***
13	Growth under vaspar seal	85	85	79	79	82	2.7
14	$CaHPO_4$ dissolution	17	17	24	26	21	4.3
15	Growth on Ca-glycerophos-phate – mannitol	84	85	90	86	86	2.5
16	White precipitate on previous medium	8	14	8	9	10	3.5
17	Starch hydrolysis	40	29	48	42	39	11.4**
18	Gelatin hydrolysis	24	49	56	57	48	29.8***
19	Proteolysis on egg yolk	7	7	18	9	10	11.1*
20	Peptonization of litmus milk	15	45	40	26	34	29.1***
21	Reduction of dye in litmus milk	36	40	65	50	48	22.6***
22	Slime produced in litmus milk	8	4	16	3	8	19.3***
23	Alkalization of litmus milk	10	13	7	8	10	3.3
24	Lecithinase	16	21	36	16	23	18.0***
25	Lipolysis of egg yolk	11	12	8	7	10	2.2
26	Lipolysis of Tween 80	49	44	60	56	52	8.0*
27	Phosphatase	31	40	38	49	40	7.1
28	Urease	44	45	31	38	40	7.2
29	Acetamidase	38	40	21	42	35	14.9***
30	Penicillin resistance (100 i.u./ml)	33	23	19	16	22	8.7*
31	Growth at 3°	55	61	70	65	63	6.1
32	Growth at 37°	38	18	9	13	19	35.9***
33	Reduction of methylene blue	26	36	23	20	27	10.3*
34	Denitrification	11	3	14	6	8	13.9**
35	Reduction of NO_3^- to NO_2^-	22	56	49	51	47	32.2***
36	Oxidase	22	79	47	55	54	80.1***
37	Growth on NH_4^+ and mixed C source	43	36	51	46	44	6.1
37[a]	Amino acids required for growth	15	11	18	16	15	2.4
38	Soil and (or) yeast extract required	42	52	32	38	42	12.9**
39	Minimal pH permitting growth = 4.0	59	44	22	30	38	37.2***
40	Minimal pH permitting growth = 4.5	19	31	51	47	37	30.7***
41	Chitinase	16	8	6	4	8	9.7** (d.f.=2)
42	Use of protocatechuic acid	37	47	28	37	38	10.2*

[a]Not included in factorization.
*Significant at 95% level.
** " " 99% "
*** " " 99.9% "

after 14 days (Katznelson and Bose 1959), using IM agar containing 1% glucose as base medium for those strains which failed to grow on the semisynthetic medium of Katznelson and Bose. Growth and production of white precipitate on mannitol – Ca-glycerophosphate agar (Riker *et al.* 1930) after 7 or 21 days. Starch hydrolysis on IM agar containing 0.2% soluble starch after 10 or 14 days. Gelatine hydrolysis on IM agar with 0.05% gelatin, flooded after 7 days with acid $HgCl_2$ solution. Peptonization, reduction of dye, slime production, and alkalization of litmus milk after 7, 14, or 21 days. Proteolysis, lecithinase reaction, and lipolysis on egg yolk-containing IM agar (Willis 1960). Lipolysis of Tween 80 on IM agar without addition of phosphate, and with increased amounts, 5 g, 3 g, and 0.1 g/l of tryptone, yeast extract, and $CaCl_2 \cdot H_2O$, respectively (Sierra 1957). Phosphatase activity noted as release of phenolphthalein after 7 days on IM agar enriched with tryptone and yeast extract as for the previous test (*TY agar*), and containing 0.01% disodiumphenolphthalein phosphate. Urease and acetamidase after 7 or 14 days on IM agar, the phosphate of which was changed to 2 g of KH_2PO_4 per liter, and to which was added 1 g/l of urea or acetamide, respectively, separately sterilized by filtration (Bühlmann *et al.* 1961). Resistance to penicillin, visible as growth after 7 or 14 days on TY agar containing 100 i.u./ml of G-penicillin, in comparison with a penicillin-free control. Growth at 3° in 21 days, and at 37° in 7 days noted in semisolid IM agar stab cultures. Reduction of methylene blue in 10 days in a liquid IM medium containing 5 p.p.m. of the dye (Katznelson and Rouatt 1957). Denitrification after 7 days and nitrate reductase activity after 3, 7, or 14 days in stab cultures in semisolid TY agar containing 1 g/l each of glucose and potassium nitrate. Denitrification was noted as gas accumulating in a tube sealed with vaspar, and the presence of nitrite with Griess-Ilosvay's reagents in a loopful of the growth in an uncovered tube. Oxidase activity in semisolid IM agar after 7 or 28 days, tested with dimethyl-*p*-phenylenediamine dihydrochloride (Sundman and Laihia 1968). Nutritional requirements, as judged from the last of three consecutive growth passages in liquid media containing (*a*) minerals, ammonia, and a mixed carbon and energy source (medium C of Sundman and Carlberg 1967); (*b*) vitamin-free casamino acids, 4 g/l in addition; or (*c*) tryptone, yeast extract, and soil extract in addition, according to media I, II, and V of Taylor (1951). Minimum pH permitting growth after 14 days in semisolid IM agar buffered with McIlwaine's phosphate citrate buffer (Sundman 1969). Chitinase after 14 days on IM agar containing partly hydrolyzed chitin, 5 g/l stratified on water agar (Gray and Bell 1963). Use of protocatechuic acid after 7 days on IM agar containing 0.1 g of the acid per liter (Sundman 1968*a*).

Factor Analytical Procedure

Standard programs were used for all treatments of data with an IBM 1620 computer of the Department of Applied Mathematics, University of Helsinki. The basic procedure was the same as previously described (Sundman and Gyllenberg 1967; Sundman 1968*b*). The correlations between each possible pair of n tests were calculated according to the test results coded for all isolates investigated. The $n \times n$ correlation matrix, which defines an n-dimensional test vector space, was condensed by factor analysis to a J-dimensional factor space ($J < n$), the vectors of which were rotated using the "varimax" method (Harman 1960). The J rotated factors, which determine the factor space, can each be interpreted in terms of the tests applied, ranked in order of test "loadings", viz. of length of the projections of the test vectors of the n-space on the J factor axes.

Each of the 467 isolates investigated was regarded as an item point in the rotated J-space common to all the isolates. The position-determining J factor values, in standard metrics, were determined for each item point. The item points defined were grouped into four populations according to the soil samples from which they were derived, and the various factors were submitted to between/within population analysis of variance, to determine their *discriminative power*. Those factors which gave insignificant between/within population variance ratios ($P \geqslant 5\%$), and hence were not effective in discriminating between populations, were not taken into account.

The position of the point centroid of each population in the selected population-discriminating factor space was determined, and the populations described by means of the corresponding factor profiles.

Finally the populations were compared in terms of the squared distance in the selected discriminative factor space between the point centroids of the populations.

Results

Primary Data

The percentual frequency of positive test results among the isolates from the four soil samples are shown in Table II. The *chi-square* figures in the last column of the table were computed from the data of the 2×4 contingency tables of positive and negative attributes in the four populations, test by test, to test the null hypothesis that the distribution of attributes was identical in the four populations studied.

According to the χ^2 figures of Table II, 27 of the attributes considered were unequally distributed in the various populations with $> 95\%$ probability. Hence, the corresponding 27 tests should be useful for a discriminative description of the populations. The best suited tests are 36 (oxidase), 39 (growth at pH 4.0), 10 (acid from maltose), 32 (growth at 37°), 3 (non-diffusible pigment), and 35 (nitrate reductase), in that order, according to the *chi-square* figures. As shown by Table II a high frequency of oxidase-positive bacteria was characteristic of the grassland soil population. The forest humus sample contained a maximum of acid-tolerant bacteria, of organisms capable of using maltose, and of those growing at 37°, whereas isolates producing non-diffusible pigment were most frequent in the field soils. The presence of nitrate reductase, like

the presence of oxidase, was a characteristic of the grassland soil isolates.

Factor Analytical Results

Before factorization, the 44 × 44 matrix of correlations between attributes was inspected to remove attributes which caused strong truistic correlations (Sundman and Gyllenberg 1967), cf. nuisance correlations of William and Dale (1965), and which lacked significant correlations with any other attribute included. On these grounds, two attributes, 2*a* (permanently prevailing rod form) and 37*a* (amino acids required for growth), were excluded, the former showing only slight correlations with other attributes ($r_{max} = 0.15$), and the latter showing only one strong correlation, namely the truistic negative correlation with attribute 37 (growth on NH_4^+ and mixed carbon source) ($r = -0.38$). The factorization of the remaining 42 × 42 correlation matrix, using maxi-

TABLE III

Extract of rotated factor matrix. First two decimals of loadings ⩾ 0.20 are given, plus signs are omitted

	Factors									
Test	1	2	3	4	5	6	7	8	9	
1			31							
2					39					
3			−21					41		
4		67								
5	76									
6	73									
7	62				−26					
8	66		−20							
9	57				29			20		
10	60				33					
11	72									
12	33				52					
13									20	
14	28						−22		−23	
15	23		26	−32						
16		54								
17									−26	
18			31		25	−20	30	29		
19								66		
20			26				34	36		
21			29	−21				45		
22										
23		38								
24		20					66			
25		35					50			
26								33		
27								47		
28									42	
29				−21					39	
30		40				22		24	26	
31				−26	−25			36		
32					61					
33		39							35	
34		26								
35			59							
36			66							
37		24		−78						
38				80				−23		
39						76			24	
40						−76				
41					30					
42		26			−25				48	
Factor variances:	3.59	1.96	1.55	1.77	1.63	1.41	1.67	1.49	1.29	Total: 16.36
% of total	21.9	12.0	9.5	10.8	10.0	8.6	10.2	9.1	7.9	

mal correlations as estimates of communality, was continued until a factor was obtained with an eigenvalue of < 0.5. This entailed extraction of 11 factors. Their cumulated factor variance covered 104% of the total estimate of communality. The further description of the populations was restricted to the first nine factors, the variance of which amounted to 99% of the total estimated communality, and 43% of the number of attributes. The eigenvalue of the ninth factor was 0.65. An extract of the rotated factor matrix of these nine factors is shown in Table III. For the sake of easy interpretation, factors 3, 4, 6, and 7 were reflected (Sundman and Gyllenberg 1967).

Interpretation of Factors (Table III)

Factor 1—Attributes connected with *carbohydrate metabolism* define this factor. In addition to attributes 5–12, concerned with acid production from various carbohydrates, attributes 14 (dissolution of $CaHPO_4$), and 15 (growth on mannitol and Ca-glycerophosphate) are the only loadings of significance.

Factor 2—Attribute 4 (diffusible pigment) is "purely" loaded, viz. the loadings of the attribute in the other factors are small in comparison. Hydrolytic esterase functions are loaded for attributes 16 (hydrolysis of Ca-glycerophosphate), 24 (lecithinase), and 25 (lipolysis on egg yolk). Penicillin resistance (30), denitrification (34), simple nutritional requirements (37), and potential ability to use aromatic compounds (42) are further attributes of importance. This might be interpreted as a *Pseudomonas* factor. It is noteworthy that only 4% of the isolates produced diffusible pigment (Table II), and hence contributed to the main loading of the factor.

Factor 3—The purely loaded attributes of nitrate reductase (35) and oxidase (36) dominate. A strong correlation between these two attributes has previously been demonstrated for soil bacteria from various sources (Sundman and Gyllenberg 1967). The other significant loadings appearing in this *oxidase – nitrate reductase factor*, including Gram-positivity (1); reduction of litmus (21); proteolytic activity on casein (20) and on gelatin (18); growth on mannitol and Ca-glycerophosphate (15); lack of ability to use xylose (8); and lack of non-diffusible pigment (3) are not easily interpreted. It is noteworthy that the Gram reaction has its strongest loading, indicating Gram-positivity, in this factor.

Factor 4—Nutritional requirements are involved. The truistic factor is created by the strong negative correlation between the mutually exclusive attributes of simple and complex nutritional requirements (37 and 38). The negative loadings indicating inability to split acetamide (29) and to grow on Ca-glycerophosphate and mannitol (15) might be reflections of the *complex nutritional demands* which dominate the factor. The inability to reduce litmus (21) and to grow at 3° (31) are not easy to explain in this connection.

Factor 5—Since the attribute of presence of endospore (2) has its maximum loading here, the factor was interpreted as a *Bacillus* factor. The major loadings indicate growth at 37° (32), fermentative glucose metabolism (12), inability to use rhamnose (7), use of saccharose (9) and maltose (10), hydrolysis of gelatin (18), inability to grow at 3° (31), failure to use protocatechuic acid (42), and ability to degrade chitin (41). According to Table II, the endospore-producing isolates were 4% of the total, and were mainly derived from the forest humus. The factor bears some resemblance to the prevailing characteristics of sporeforming rods from an acid pine forest soil described by Goodfellow *et al.* (1968).

Factor 6—The heavy opposite loadings of the mutually exclusive attributes of pH tolerance (39–40) dominate. The combination of acid tolerance (39), resistance to penicillin (30), and inability to hydrolyze gelatin (18) suggest the interpretation: *ecological factor of acid environment.*

Factor 7—The significant loadings are restricted to attributes of proteolysis (18–20) and of esterase activity (24–25). A slight negative loading of Ca-phosphate dissolution (14) completes the list. A connection between *proteolytic and esterase activities* was previously demonstrated with pseudomonads by Gyllenberg and Eklund (1967), and with random bacterial isolates from various soils by Sundman (1968*b*), who pointed out the possible parallelism to known pancreas proteinases with esterase function. Quadling (1967) recognized a similar attribute complex (No. 12) with cultures of named Gram-negative rods and *Bacillus* species.

Factor 8—This distinct and clearly defined factor is dominated by attributes on non-diffusible pigment (3), esterase activities (26–27),

proteolytic abilities (18 and 20), and glucosidase activity (9). Resistance to penicillin (30), growth at 3° (31), and simple nutritional requirements, manifested as a negative loading of attribute 38, are further involved. Apart from the pronounced loading for reduction of litmus (21), the factor is similar to the *factor of pigment-correlated hydrolytic abilities* extracted by Sundman (1968*b*) from data on random soil isolates, and by Gyllenberg and Eklund (1967) under the name of "food deterioration factor" from data on psychrophilic pseudomonads.

Factor 9—Use of protocatechuic acid (42) dominates. Correlated attributes are urease (28), and acetamidase (29), the former being purely loaded, resistance to penicillin (30), tolerance to acid (39), reduction of methylene blue (33), independence of oxygen for growth (13), and inability to hydrolyze starch (17) or to dissolve $CaHPO_4$ (14). The factor is diffuse and difficult to interpret. Protocatechuic acid utilization points to metabolism of aromatic compounds, and the restricted need for oxygen, together with the ability to mobilize amide nitrogen, suggested the hypothetical denomination of a *humification factor.*

Qualitative Description of Populations by Selected Factor Profiles

The nine factors described in the previous paragraph determine an orthogonal 9-space. The position of the item points of the single isolates in this space were computed. The result of the calculation, viz. the 9 × 467 factor values, is condensed in Table IV, which shows the position, in terms of nine factor values, of the point centroids of the four populations. These were taken as estimates of the position in the 9-factor space of the entire set of isolates making up a population. The 9 × 467 matrix of factor values of the single isolates was submitted to factorwise analysis of variance of between/within population variation. The result is given as $F_{(3, 463)}$ figures in Table IV. Six of the nine factors have highly significant variance ratios ($P < 0.1\%$), indicating greater variation between than within populations. These selected factors (3, 4, 5, 6, 8, and 9), covering 56% of the factor variance considered, are highly effective in discriminating the populations studied, whereas the other three factors, covering 44% of the factor variance, are determined by attributes with distributions which are hardly at all or only slightly significantly influenced by the ecotype studied.

By means of the six ecologically discriminating factors selected, the four bacterial soil populations can be characterized as shown in Fig. 1. The figure is a graphical presentation of the factor values of Table IV, and determines the position of the four population centroids in the selected 6-space. The factors in the profiles or histograms of Fig. 1 are presented from left to right in order of decreasing discriminating efficiency according to the $F_{(3, 463)}$ values. To understand the information in Fig. 1, it is necessary to consult the rotated factor matrix (Table III) and the interpretation of factors in the results above. According to the factor profiles of Fig. 1, the oxidase – nitrate reductase factor (factor 3) has a high estimate for the grassland soil population, a low estimate for the forest humus population, and medium estimates for the field soil populations. This implies that

TABLE IV

Factor values, in standard metrics, of the point centroids of the populations investigated and the population-discriminating efficiency of the factors ($F_{3, 463}$) according to analysis of variance. The means of the factor values were standardized to 500, their deviation to 100

	Factors								
Population	1	2	3	4	5	6	7	8	9
Forest humus	518	489	445	491	547	539	495	474	516
Grassland	489	515	526	528	500	509	495	495	523
Field, site 1	504	499	507	478	476	474	521	513	467
Field, site 2	496	491	505	493	487	483	487	516	491
$F_{(3, 463)}$	2.06	2.51	21.07***	8.12***	14.84***	12.37***	3.68*	6.13***	14.25***

*Significant at 95% level.
***Significant at 99.9% level.

isolates which showed a positive response in those tests which are strongly positively loaded in factor 3 were frequent in grassland soil, sparse in forest humus, and medium frequent in field soil. A scrutiny of Table III and the primary data of Table II confirm this. The factor profile of the forest soil population is characterized by high estimates of the *Bacillus* factor (factor 5) and of the factor of acid environment (factor 6), whereas high estimates of the humification factor (factor 9) and of the factor of complex nutritional demands (factor 4) are typical of the grassland soil population. The factor of pigment-correlated hydrolytic ability (factor 8) has its highest estimates in the two field soil population profiles.

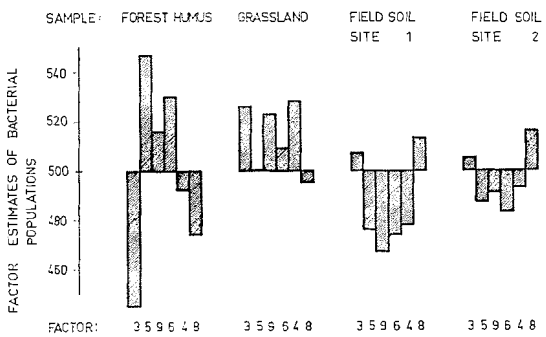

FIG. 1. Factor profiles, in standard metrics, of bacterial populations from four soil samples. The result was based on a 9×42 rotated factor matrix, taking into account only the six factors with values which showed significantly ($P < 0.1\%$) greater variation between populations than within populations, according to $F_{(3, 463)}$ distribution. The profiles determine positions of point centroids of populations in the 6-factor space. The description of factors is as follows.

No.	$F_{(3, 463)}$	Interpretation
3	21.07	Oxidase – nitrate reductase
5	14.84	Bacillus
9	14.25	Humification
6	12.37	Acid environment
4	8.12	Complex nutritional demands
8	6.13	Pigment-correlated hydrolytic abilities

TABLE V

Squared distances (d^2) between pairs of populations according to position of population means in the selected discriminating 6-factor space, as defined by the profiles of Fig. 1. Relative figures indicating $d^2 \times 10^{-2}$ are given

	Forest humus	Grass-land	Field, site 1	Field, site 2
Forest humus	0	115	172	127
Grassland	115	0	81	40
Field, site 1	172	81	0	10
Field, site 2	127	40	10	0

Comparison of the Populations by d^2-Statistics

The visual inspection of the profiles in Fig. 1 gives the impression that the two duplicate field soil populations are more similar to each other than to the other populations. A measure for numerical estimation of the differences between item points in a factor space is the squared distance between the points (Grieg-Smith 1964; Williams and Dale 1965; Skyring and Quadling 1969). The squared distances (d^2) between all possible pairs of population centroids in the discriminating six-factor space was computed according to the positions determined by Fig. 1. The result is shown in Table V. The relative distances in the matrix of the table were obtained by dividing d^2 by 100. The similarity of the populations from the two duplicate field soil sites is evident ($d^2 \times 10^{-2} = 10$) in comparison with the differences between populations from the forest humus field and from soil sites ($d^2 \times 10^{-2} = 172$, and 127, respectively). The grassland microbial population is closer to the field soil populations ($d^2 \times 10^{-2} = 81$, and 47, respectively) than to the forest humus population ($d^2 \times 10^{-2} = 114$). The close resemblance of the two duplicate populations is still more convincing when it is borne in mind that the result was obtained with a numerical method, the aim of which was to detect differences between the populations studied.

Discussion

Among the nine factors studied, those three (factors 1, 2, and 7) which, according to Table IV, did not contribute significantly to differentiation of the populations from the various soils, covered 44.1% of the total variance of the nine factors (Table III). Roughly one half of this variance (21.9%) is contributed by the factor of carbohydrate utilization (factor 1). Though tests on acid production from carbohydrates have been of fundamental significance in identification and taxonomic work, it seems that their usefulness for description of bacterial soil populations might be restricted. The same conclusion was drawn from the *chi-square* figures of Table I. Of the attributes for carbohydrate utilization, only those concerning aerobic use of disaccharides and fermentative

use of glucose showed significantly uneven distribution among the bacterial populations studied.

The estimate of the *Bacillus* factor (factor 5) is highest for the forest humus population. Several authors have shown that sporeforming rods are frequent in acidic peaty soils, and in forest soils (Gyllenberg *et al.* 1954; Jensen 1963; Holding *et al.* 1965; Goodfellow *et al.* 1968). The reason for the occurrence of bacilli in acid soil remains obscure. No significant correlation was found in the present material between the presence of endospore and the tolerance of an acid environment ($r_{\text{spore/tolerance of pH 4.0}} = 0.043$, and $r_{\text{spore/tolerance of pH 4.5}} = 0.099$).

The ability to dissolve chitin was most frequent among the forest isolates. Gray and Bell (1963) found that the decomposition of chitin in an acid soil is brought about by fungi and motile Gram-negative bacteria. In the present material no significant correlation could be established between ability to decompose chitin and the Gram reaction ($r_{\text{chitinase/Gram}} = 0.078$). In the factor matrix (Table III), the *Bacillus* factor (factor 5) is positively loaded by the chitinase attribute (41). On the other hand, the *Pseudomonas* factor (factor 2), which is dominated by a high loading for diffusible pigment, has no significant loading of chitinase activity. Factor 8 of pigment-correlated hydrolytic ability has a slight loading of chitinase activity (0.168), not indicated in Table III. Hence it seems likely that *Bacillus* species, and to a lesser extent hydrolytic pigmented bacteria, might be involved in chitin breakdown in the soil. For the role of *Bacillus* in chitin breakdown in forest soil see Gray and Baxby (1968).

According to the *chi-square* values of Table II, the attribute of oxidase activity (36) is most effective for discrimination of the various bacterial populations ($\chi^2 = 80.1$). Oxidase and nitrate reductase are strongly correlated, and purely loaded in factor 3, which accordingly has maximal discriminative efficiency (cf. Table IV). The significance of the oxidase attribute for the description of bacterial populations of soil origin has repeatedly been pointed out (Sundman and Gyllenberg 1967; Sundman 1968*b*). The estimate of an oxidase and nitrate reductase factor was significantly higher for a population from fertile garden compost than for populations from acid forest soils, and from decaying sawdust (Sundman 1968*b*). In the present material the oxidase – nitrate reductase factor dominates the grassland soil population, and has its lowest estimate for the population of acid forest humus (Table IV). According to the experience of the author, the oxidase attribute, which is strongly correlated with the nitrate reductase attribute ($r = 0.49$ in the present material, and $r = 0.64$ in the previous material (Sundman 1968*b*)) might be a useful tool for bacteriological evaluation of soil fertility.

The repeatedly observed correlation of production of non-diffusible pigment and of hydrolytic capability among bacteria from diverse sources (Gyllenberg 1965; Gyllenberg and Eklund 1967; Sundman 1968*b*) which in the present material is condensed into factor 8 merits special attention. It is possible to imagine that this factor reflects a genetic or enzymatic combination present in a wide variety of bacteria. A further study of the taxonomic relationships between isolates which have strong values of this factor is needed to elucidate the question.

It has repeatedly been stressed that there is a need for methods which would permit estimation of variation within the bacterial soil populations (Rovira and Brisbane 1968; Skyring and Quadling 1969). The results presented in this paper seem to indicate that in spite of the difficulties inherent in descriptive soil microbiology, such as the small part of the soil population accessible to the present isolation technique, the factor analytical approach gives possibilities for meaningful comparison of bacterial populations, even without any endeavor at clustering or denomination of individual isolates.

Acknowledgment

This investigation was financially supported by grants from the National Research Council for Agriculture and Forestry (Finland), which is gratefully acknowledged. Thanks are due to Mrs. Ritva Boman and Mrs. Sirpa Ilovaara for skilful technical assistance.

BÜHLMANN, X., W. A. VISCHER, and H. BRUHIN. 1961. Identification of apyocyanogenic strains of *Pseudomonas aeruginosa*. J. Bacteriol. **82**: 787–788.

GOODFELLOW, M., I. R. HILL, and T. R. G. GRAY. 1968. Bacteria in a pine forest soil. *In* The ecology of soil bacteria. An international symposium. *Edited by* T. R. G. Gray and D. Parkinson. Liverpool University Press. pp. 500–515.

GRAY, T. R. G., and P. BAXBY. 1968. Chitin decomposition in soil. II. The ecology of chitinoclastic micro-

organisms in forest soil. Trans. Brit. Mycol. Soc. **51**: 293–309.

GRAY, T. R. G., and T. F. BELL. 1963. The decomposition of chitin in an acid soil. *In* Soil organisms. *Edited by* J. Doeksen and J. van der Drift. North-Holland Publishing Company, Amsterdam. pp. 222–229.

GRIEG-SMITH, P. 1964. Quantitative plant ecology. 2nd ed. Butterworth & Co. (Publishers) Ltd., London.

GYLLENBERG, H. 1964. An approach to numerical description of microbial populations. Ann. Acad. Sci. Fenn. Ser. A (IV) Biol. **81**: 4–23.

——— 1965. Character correlation in certain taxonomic and ecologic groups of bacteria. A study based on factor analysis. Ann. Med. Exp. Fenn. **43**: 82–90.

GYLLENBERG, H., and E. EKLUND. 1967. Evaluation of character correlation patterns in psychrophilic pseudomonads. Ann. Acad. Sci. Fenn. Ser. A (IV) Biol. **113**: 3–19.

GYLLENBERG, H., P. HANIOJA, and U. VARTIOVAARA. 1954. Observation on the composition of the microbial population in some virgin soils. Acta Forest. Fenn. **62**: 1–31.

HARMAN, R. 1960. Modern factor analysis. The University of Chicago Press, Chicago.

HILL, L. R., L. G. SILVESTRI, P. IHM, G. FARCHI, and P. LANCIANI. 1965. Automatic classification of staphylococci by principal-component analysis and a gradient method. J. Bacteriol. **89**: 1393–1401.

HOLDING, A. J., D. A. FRANKLIN, and R. WATLING. 1965. The microflora of peat-podsol transitions. J. Soil Sci. **16**: 44–59.

HUGH, R., and E. LEIFSON. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol **66**: 24–26.

JENSEN, V. 1963. Studies on the microflora of Danish beech forest soils. III. Properties and composition of the bacterial flora. Zentralbl. Bakteriol. (II) **116**: 593–611.

KATZNELSON, H., and B. BOSE. 1959. Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere, and nonrhizosphere soil. Can. J. Microbiol. **5**: 79–85.

KATZNELSON, H., and J. W. ROUATT. 1957. Studies on the incidence of certain physiological groups of bacteria in the rhizosphere. Can. J. Microbiol. **3**: 265–269.

MORONEY, M. J. 1962. Facts from figures. Penguin Books, Harmondsworth. p. 258.

PRASAD, M. 1968. Vergleichende Untersuchungen über verschiedene Methoden zur quantitativen Erfassung der Bodenmikroflora. Zentralbl. Bakteriol. (II) **122**: 341–356.

QUADLING, C. 1967. Evaluation of tests and grouping of cultures by a two-state principal component method. Can. J. Microbiol. **13**: 1379–1400.

RIKER, A. J., W. M. BANFIELD, W. H. WRIGHT, G. W. KEITT, and H. E. SAGEN. 1930. Studies on infectious hairy root of nursery apple trees. J. Agr. Res. **41**: 507–540.

ROVIRA, A. D., and P. G. BRISBANE. 1968. Numerical taxonomy and soil bacteria. *In* The ecology of soil bacteria. An international symposium. *Edited by* T. R. G. Gray and D. Parkinson. Liverpool University Press. pp. 337–350.

SIERRA, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **23**: 15–22.

SKYRING, G. W., and C. QUADLING. 1969. Soil bacteria: Comparison of rhizosphere and nonrhizosphere populations. Can. J. Microbiol. **15**: 473–488.

SUNDMAN, V. 1968*a*. A simple plate test for the determination of protocatechuic acid utilization by soil bacteria. Can. J. Microbiol. **14**: 88–89.

——— 1968*b*. Characterization of bacterial populations by means of factor profiles. Acta Agr. Scand. **18**: 22–32.

——— 1969. The pH tolerance of soil bacteria. Finska Kemistsamfundets Medd. **78**: 32–37.

SUNDMAN, V., and G. CARLBERG. 1967. A comment on the nutritional grouping of soil bacteria. Can. J. Microbiol. **13**: 565–568.

SUNDMAN, V., and H. GYLLENBERG. 1967. Application of factor analysis in microbiology. I. General aspects on the use of factor analysis in microbiology. Ann. Acad. Sci. Fenn. Ser. A (IV) Biol. **112**: 3–32.

SUNDMAN, V., and K. LAIHIA. 1968. The occurrence of methoxy-*p*-benzohydroquinone-oxidizing ability among soil bacteria and its correlation to other phenol oxidase activities. A factor analytical approach. Finska Kemistsamfundets Medd. **77**: 142–152.

TAYLOR, C. B. 1951. The nutritional requirements of the predominant bacterial flora of the soil. Proc. Soc. Appl. Microbiol. **14**: 101–111.

WILLIAMS, W. T., and M. B. DALE. 1965. Fundamental problems in numerical taxonomy. Advan. Bot. Res. **2**: 35–68.

WILLIS, A. T. 1960. The lipolytic activity of some clostridia. J. Pathol. Bacteriol. **80**: 379–390.